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Year: 2013

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## **Diagnostics of endurance performance on the level of gene expression**

Flück, Martin

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DOI: <https://doi.org/10.1016/j.orthtr.2013.07.014>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-90974>

Journal Article

Accepted Version

Originally published at:

Flück, Martin (2013). Diagnostics of endurance performance on the level of gene expression. *Sport-Orthopädie - Sport-Traumatologie*, 29(3):203-213.

DOI: <https://doi.org/10.1016/j.orthtr.2013.07.014>

**Diagnostics of endurance performance on the level of gene expression**

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## Summary

Physiological measures of exercise performance provide reference points for physical health and fitness. They allow to evaluate the progression of improvements in work capacity with training in the active sportsman or the injured with rehabilitation. Tests are now established in many Sports Clinics to document task-specific exercise performance. Major developments in the area of the molecular health sciences highlight options to reinforce current performance testing with molecular diagnosis. In the following, a personal view of the perspectives of exercise testing at the molecular level is given with respect to endurance performance. The case is developed that local, biopsy-based measures of the transcript response of exercised muscle to endurance work may be used to estimate specificity, pace, and possibly magnitude of adaptation with repeated endurance stimuli. This expression profiling of muscle's adaptive response to an exercise stimulus complements non-invasive, genomic methodologies that have identified the association of exercise performance with modifications in heritable elements (gene polymorphisms). Research applying these tools highlights the possibility that the molecular analysis of sample collected with minimally invasive methodology from peripheral muscle tissue and blood serum can enhance the diagnostic power of current physiological tests, and lend to a future use in predicting the progression and variability of endurance performance with training.

*Keywords:* Skeletal muscle, biopsy, serum, gene, prognosis

1 Classical exercise physiology has provided valuable tools to quantify maximal work capacity  
2 over a large spectrum of power output and forms of physical activity. This covers lab-based  
3 tests measuring force production and power output of single, isolated contractions, as well as  
4 field tests assessing energy expenditure of repeated contractions in relation to discipline  
5 specific performance (table 1). A major benefit of these tests is that the conversion of  
6 metabolic energy into mechanical output is assessed in an integrated manner in a whole body  
7 context.  
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13 *Limitations in current performance testing* - A major drawback of functional tests into  
14 work capacity is that these are often too coarse to resolve improvements or bottlenecks in the  
15 processes that conspire to set maximal performance. Maximal performance depends on a  
16 number of interrelated biological pathways/organ systems (Fig. 1). However these do only  
17 demonstrate minute adjustments with each bout of exercise. In consequence standard  
18 performance tests such as ergospirometry do not typically resolve the individual processes [1-  
19 3]. In consequence they cannot provide specific answers on functional improvements before  
20 an important period of training has passed. This limits their use in the selection and fine-  
21 tuning of training and probably explains why experienced coaches prefer to explore  
22 psychological options to maximize performance through motivating the recruitment of  
23 functional reserves in Athletes. Recent additions to exercise testing involve the assessment of  
24 genetic constitution through the measure of gene variants, or polymorphisms, being associated  
25 with performance-related phenotypes. According to leading experts, however, current genetic  
26 testing has zero predictive power on talent identification [4].  
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40 *Endurance training* – The ultimate purpose of endurance training is to increase the  
41 duration during which force production of repeated contractions can be maintained.  
42 Classically this is achieved through the completion of a protocol of repeated exercise sessions  
43 with a high number of contractions at low load. Thereby the distinction to power sports is  
44 typically drawn based on soft parameters respective to the individual maximal values of  
45 power output during a workout and the duration during which this can be performed (such as  
46 a VO<sub>2</sub>max test).  
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54 Improvements in endurance performance of running type exercise for instance can be  
55 assessed via the measure of running economy, peak velocity and time-to-exhaustion in time  
56 trials [5, 6]. The adequacy of these measures is amply demonstrated for elite athletes [7-9] but  
57 they are rarely performed in routine diagnosis. At best maximal oxygen uptake (VO<sub>2</sub>max) is  
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1 assessed in an incremental test, which allows, when accompanied with measures of  
2 mechanical work, to estimate the economy of a given exercise. VO<sub>2</sub>max, however, is a steady  
3 state measure of maximal exercise performed at relatively short duration. This parameter,  
4 while reporting maximal aerobic performance, does not necessarily document fatigue  
5 resistance, which is probably more relevant to characterize performance of long distance  
6 events, which rely on the capacity to maintain substrate metabolism and metabolic stores.  
7 This distinction is also important because the improvement in endurance performance with  
8 training is more pronounced for factors reflecting the time-to-exhaustion or velocity at a high  
9 metabolic strain, than for VO<sub>2</sub>max, which typically only improves moderately [7, 8].  
10 Towards a representative diagnostics of endurance performance it therefore appears sensitive  
11 to target the tests as close as possible to the muscle groups being trained and quantify both the  
12 maximal capacity for aerobic work and the time-to-exhaustion.  
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23 *Myocellular underpinning of exercise performance* – As pointed out in a number of  
24 research papers, measures comparing structure-function relationships in the effector organ of  
25 mechanical work, skeletal muscle, can reveal unprecedented detail on the features that set the  
26 endurance training state. For instance, muscles of endurance-trained subjects have been  
27 shown to differ to un-trained subjects in terms of muscle size and fat content, slow fiber type  
28 composition and myocellular constituents of lipid metabolism [10, 11]. The theme of these  
29 adaptations is the promotion of aerobic substrate pathways that increased the economy of  
30 muscle contractions.  
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39 Performance in most individual sports disciplines has its foundation in two, partially  
40 exclusive traits that reflect the maximization of power output or fatigue-resistance of a motor  
41 task, i.e. strength vs. endurance (reviewed in [12]). Skeletal muscle contributes in a specific  
42 way to the furthering of both traits with training. It has been appreciated that this is mostly  
43 explained by the composition in motor units [13]. Three major motor unit types are described  
44 based on electrophysiological measures of contractile and metabolic characteristics, i.e. a  
45 slow-fatigue-resistant, a fast-fatigue-resistant, and a fast-fatigable type [14]. They can be  
46 mapped with a histological or immunological analysis of sections from muscle samples for  
47 mitochondrial enzymes (i.e. succinate dehydrogenase, cytochrome c oxidase, nicotinamide  
48 adenine dinucleotide-dehydrogenase, alpha glycerophosphate dehydrogenase) and the major  
49 myosin isoforms that confer motor units their typical contractile characteristics (i.e. I, IIA,  
50 IID/X)[15]. For the comprehensive characterization, the distribution and cross sectional area  
51 of fiber types fiber is then determined by the quantification of area or volume content [16].  
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1 This characterization can be complemented with fiber specific measures of capillary density  
2 to reveal the local capacity for substrate supply [17, 18].  
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4 Owing to the different metabolic and mechanical characteristics of motor unit types,  
5 the optimization of power and endurance is reflected at the level of muscle, and muscle fiber,  
6 composition [19]. For instance, endurance athletes demonstrate a high content of slow-type  
7 fibers which contract more economically than fast-type fibers, and increased capillarity along  
8 with elevated volume density of mitochondria in the individual muscle fibers [17]. By  
9 contrast increased muscle strength and power, is associated with a high content in fast type  
10 muscle fibers and elevated myofibrillar volume density, and a concomitantly reduced density  
11 of mitochondria owing to their dilution in a larger volume. A major drawback of these  
12 myocellular measures is that they are rarely carried out due to the lack of medical  
13 infrastructure being required to collect, and characterize, muscle samples.  
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24 *Exercise-induced muscle malleability* - Skeletal muscle is known to demonstrate a  
25 large degree of cellular plasticity to work-related stimuli compared to other tissues being  
26 recruited with exercise (Fig. 1). For instance, local aerobic capacity in skeletal muscle of  
27 untrained subjects can nearly double after 6-weeks of bicycle-type endurance training. By  
28 contrast, the contribution of this adaptation to whole body aerobic capacity is curtailed to a  
29 level of a few percent, only [1] and this is probably not always be resolved at the level of  
30 VO<sub>2</sub>max with ergospirometry. It follows that the characterization of muscle's aerobic  
31 capacity can provide a valuable complement for classical physiological tests to estimate  
32 endurance capacity and its malleability with training.  
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42 The suitability of muscle-targeted tests is illustrated by increases in the volume density  
43 of capillaries, mitochondria and intramyocellular lipid stores in the knee extensor muscle,  
44 *vastus lateralis*, after 6-weeks of a training paradigm on a stationary bicycle composed of  
45 thirty, half-an-hour-exercise sessions at the considerable intensity of 65% of peak aerobic  
46 power output. These adaptations are corroborated at the level of protein by an increased  
47 content of mitochondrial proteins and factors involved in glucose metabolism (Fig. 2). With  
48 continuation of the training stimulus the adjustments may further progress [10] but the extent  
49 of mitochondrial plasticity is generally reduced in extent in relation to the training state.  
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57 *The molecular approach* - With the introduction of molecular biology, the mechanism  
58 underpinning adaptations of muscle organelles to endurance training have been resolved in  
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unprecedented detail. This has been demonstrated through the quantification of adjustments in biochemical processes and the underlying gene expression in recruited muscle groups.

Gene expression is the process whereby genome-encoded information instructs the synthesis of proteins that constitute the building blocks of cellular organelles. In this process transcripts of single genes (messenger ribonucleic acids, mRNAs) provide templates for the translation of the encoded information into proteins (Fig. 3A). For instance the synthesis of mitochondrial proteins in *vastus lateralis* muscle is nearly three-fold increased within the first 4 hours after bicycle-type endurance exercise in untrained subjects [20, 21]. This response of mitochondrial protein synthesis is preserved in the trained state and is paralleled by transient elevations in transcript levels for mitochondrial factors 3-8 hours after a single bout of bicycle-type endurance exercise [22, 23]. In consequence it is thought that the improvement in local aerobic capacity with endurance training results from the accumulation of micro-adaptations in mitochondrial protein synthesis, following bursts in transcript expression, with repetition of the exercise stimulus [22, 24].

The molecular measures emphasize that transcript expression post exercise specifies the remodeling response to training. For instance it is found that the response of protein synthesis in untrained muscle to single bicycle-type endurance exercise is rather unspecific and concerns both myofibrillar and mitochondrial protein [20]. By contrast in the endurance trained-state, when mRNA templates for the synthesis of mitochondrial proteins are broadly increased, protein synthesis of pooled mitochondrial proteins, only, is specifically elevated. This is reflected by the reactivity of transcript expression after both acute endurance exercise and endurance training. This concerns gene ontologies associated with processes of aerobic metabolism such as fatty acid transport, mitochondrial beta-oxidation and respiration [25]; known to be collectively improved with endurance training (Fig. 3B). As well, in the trained state myofibrillar protein synthesis is blunted in line with moderate alterations in transcript levels for myogenic processes. This response distinguishes to transcript alterations seen after endurance exercise under a high-load complement [26, 27]. The observations support the view that the accumulation of mRNA templates with the repeated impact of endurance exercise specifies the set of proteins being synthesized with endurance training [21]. This view is supported by the significant linear correspondence between transcript expression of lipid metabolism and corresponding organelles, mitochondria and intramyocellular lipids, in an endurance-trained state [22]. Collectively, the findings indicate that measurements of the

transcript response can be used to estimate specificity, pace, and possibly magnitude of adaptation with the repetition of an endurance stimulus.

*Resolving the effect of training paradigms* - The molecular characterization of muscle's response to exercise appears particularly useful to develop and consolidate endurance-training paradigms. Such an example is endurance training in hypoxia for which proof of efficiency is debated. We have shown that the co-stimulus of hypoxia promotes a specific response of mitochondrial processes to bicycle-type endurance work. The characterization of muscle ultrastructure emphasizes that a volumetric increase in subsarcolemmal mitochondria is a hallmark of endurance-type training under lowered oxygenation [28]. Subsarcolemmal mitochondria localize in the neighborhood of capillaries and are thought to improve substrate supply to working muscle and constitute a possible bottleneck when exercising under the constraint of hypoxia. This is given because the latter environmental factor lowers the capacity for economic energy production via aerobic processes in mitochondria through the reduction of arterial oxygen saturation.

The observed muscle adjustments to bicycle type endurance training in hypoxia are corroborated by findings on the effect of interval-type endurance training based on running. These show that expressional adaptations of metabolic processes are associated with an enhanced efficiency of mitochondrial energy production and improved coupling of energy transfer from mitochondria to the myofibrillar motor via the creatine kinase shuttle [2, 29]. In this case an increase in transcripts for the facilitative glucose transport protein 4, mono-carboxylate transporter 1 and carbonic anhydrase 3 are correlated with changes in time-to-exhaustion after intermittent hypoxia training. The few examples emphasize that molecular measures tackling adaptations in metabolic pathways can expose the tuning of muscle performance by endurance training paradigms.

Lately we have extended our investigation on hypoxia-regulated adaptations in gene transcript expression with endurance exercise by a broad characterization using microarrays. The measures identify that transcript level alterations post-exercise are correlated with changes in arterial oxygenation and serum lactate. Thereby it is found that the larger the lactate concentration in serum, the higher the level alteration in transcript expression [28]. This observation suggests that at the muscle level the disagreement on the effect of added hypoxia may be related to differences in metabolic strain achieved during exercise [30].



1 A major theme when assessing functional and structural adjustments of skeletal  
2 muscle to endurance exercise is that the magnitude of phenotypic malleability is reduced in a  
3 trained-state. For instance the increase in mitochondrial volume density in *vastus lateralis*  
4 muscle of untrained subjects can amount to a mean accumulation rate of mitochondria per  
5 muscle structure of 0.1% per exercise session (as calculated from an increase in mitochondrial  
6 volume density from 3 to 6% over 30 exercise sessions). By contrast, mitochondrial density  
7 does rarely increase above 10% even after years of endurance training with hundreds of  
8 exercise sessions. From this it is calculated that the rate for the accumulation of mitochondrial  
9 volume may drop to 0.01% per total muscle volume and session in a trained state. Meanwhile,  
10 the increase in synthesis of mitochondrial protein in muscle persists in the trained state [20].  
11 This implies that degradation of mitochondrial protein post exercise is considerably increased  
12 in an endurance-trained state and blunts the synthesis in mitochondrial protein [21, 31]. The  
13 observations suggest that the plateau in adaptation of local aerobic capacity by mitochondrial  
14 biogenesis reflects to a considerable degree increased turnover of muscle [21].  
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26 In this regard, caution should apply when assessing regulatory mechanism in elite  
27 athletes because limited information exists on muscle adaptation in this population. For  
28 instance, we identify that elite runners do not typically increase mitochondrial gene  
29 expression with a further endurance-training paradigm as seen in untrained subjects [2].  
30 Possibly this reflects the reduced scope for adaptation in aerobic metabolism after years of  
31 endurance training. By contrast the inclusion of an interval-type running paradigm under  
32 hypoxia increases transcript expression of metabolic factors after 6 weeks in line with  
33 functional improvements in time-to-exhaustion [2]. The observations suggest that gene  
34 expression may serve to replenish proteins that are lost with work-related wear-and-tear of  
35 muscle tissue. Possibly this reflects changes in set points of regulation as has been pointed out  
36 in a cohort of professional cyclists [3].  
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48 *A personalized approach to endurance training* - Variability in the response to  
49 exercise has always been a confounder in cohort studies, which was accepted as biological  
50 noise inherent to such kind of studies [32]. The early seminal paper of Petit and Klissouras  
51 pointed out the contribution of hereditary factors to the experimentally observed variability in  
52 VO<sub>2</sub>max [33]. It was however only relatively recently, with the sophistication of genetic  
53 methodology, that research on the role of hereditary factors in regulating exercise  
54 performance has gained in importance [34]. Genetic predisposition is now understood to  
55 importantly modify the chances to excel in a given Athletic Discipline.  
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1 The power of a genomic approach has been pointed out in a yearly series of papers by  
2 Bouchard and colleagues on the ‘Human Gene Map for Performance and Health-related  
3 Fitness Phenotypes’. These articles emphasize the biological relevance of gene x environment  
4 interactions for the two major traits of human performance (i.e. strength and endurance; [12,  
5 35]). Two recent reviews are worth mentioning in this regard as they focus on genetic studies  
6 in athletic cohorts. Pitsiladis and colleagues comment that over 200 small nuclear  
7 polymorphisms (SNPs) were found to be associated with physical- performance traits, and  
8 over 20 SNPs were associated with elite athletic status [4]. However, these authors conclude  
9 that ‘current genetic testing has zero predictive power on talent identification and should not  
10 be used by athletes, coaches or parents’. In their outlook on the future of Sports Genetics, they  
11 comment that beside the R577X mutation in the actinin 3 gene (ACTN3) and an I-allele  
12 insertion polymorphism, in the gene for angiotensin converting enzyme (ACE) ‘the vast  
13 majority of candidate genes for sporting performance discovered to date are not the key  
14 candidates seriously implicated in the phenotypes of interest’. These authors argue that ‘new  
15 approaches involving large, well-funded consortia and utilizing well-phenotyped large cohorts  
16 and genome-wide technologies will be necessary for meaningful progress to be made.’ In a  
17 modified pronunciation of this theme, the review by Bouchard and colleagues on Advances in  
18 Exercise, Fitness, and Performance Genomics comments on the identification of quantitative  
19 trait loci on chromosome 13q12 that explain 20% of the variance in training-induced changes  
20 in submaximal exercise capacity i.e. 60% of VO<sub>2</sub>max [36]. Distinct genome regions, holding  
21 candidate genes with muscle relevant functions, were mapped, but the responsible gene  
22 sequence was not identified. These authors propose that ‘new genomic targets should be  
23 further investigated to establish their true relevance for targeted exercise recommendations in  
24 the context of personalized exercise medicine’.

25 Owing to the role of muscle plasticity in the enhancement of exercise performance it is  
26 perceived that monitoring the molecular processes, such as transcript expression, that are set  
27 in motion in recruited skeletal muscle post exercise is a critical step towards the development  
28 of personalized, effective training programs (reviewed in [37]). Towards this end we recently  
29 pointed out that exercise-induced transcript expression provides a mechanistic connection  
30 between heritable factors and inter-individual variability in the phenotypic response to  
31 endurance exercise [38]. The investigation shows that the polymorphism for a 287bp gene  
32 insertion in intron 16 (the I-allele) of the gene for the producer of the major vasoconstrictor  
33 angiotensin 2, angiotensin-converting enzyme (ACE), affects plasticity of aerobic metabolism  
34 in skeletal muscle. This gene variant is the prototype for gene-exercise phenotype associations

[36, 39]. It was originally identified after a search of genetic elements conferring risk to coronary hypertension and stroke and is understood to occur in a frequency of nearly 50% in Caucasian populations [40]. Specifically, the presence of the I-allele is associated with 2-fold larger local improvements in subsarcolemmal mitochondria and intramyocellular lipids in knee extensor muscle compared to subjects being homozygous for the absence of the I-allele [38]. The latter genotype holds the D-allele, which is characterized by the absence of a control sequence known to reduce ACE gene transcript levels. We identify that the varying trainability in local aerobic capacity in function of the I-allele relates to differences in serum angiotensin 2 levels post exercise (data not shown). Lately we confirmed the functional relevance of ACE-genotype modulated regulation of endurance performance during the field test of a marathon [41] by demonstrating elevated serum glucose levels and finishing times in ACE-DD genotypes. This indicates the contribution of exercise-induced vasodilatation and substrate supply to the effect of the ACE I/D polymorphism on endurance performance.

These observations provide a first mechanistic explanation on the contribution of muscle tissue to the documented association between the homozygous presence, and absence, respectively, of the ACE I-allele with the two extremes of human performance, i.e. endurance and power [34]. The findings allow conclusions on the predisposition for endurance sports and provide clues as how to tailor exercise interventions for subjects with a normally reduced response in aerobic fitness to endurance training. Given that the ACE I-allele occurs in near every other subject in White Caucasians, our observations may be applicable to a considerable proportion of the general public in the Western hemisphere.

*Translating basic sciences in diagnostic practice* – Research investigations highlight the feasibility of assessing endurance performance based on the characterization of transcript expression in muscle biopsies (Fig. 3B). Transcript expression can be assessed with a few milligrams of muscle material using standard methods of molecular biology. Sampling can be achieved under local anesthesia with fine needle biopsies from defined portions of muscle tissue. The tissue is then processed to isolate, and reverse-transcribe, the labile mRNAs with commercial kits to reveal stable coding deoxyribonucleic acids (cDNA). These can then be subjected to a number of techniques such as polymerase chain reaction or microarray to assess single up to thousands of gene transcripts in one assay. These methods are sensitive and allow assessing gene transcript regulation in tissue in as little material as representing a few dozen, capped muscle fibers in a biopsy, only.

Two main areas are identified which remain to be addressed to apply molecular diagnostics to a larger audience: Firstly this concerns the collection of tissue sample. While it is appreciated that muscle quality can be characterized based on a microbioptic sample, not all sportsmen interested in this knowledge will be comfortable with the idea of being subject to this ambulatory gesture. An alternative may be the sampling of blood serum as it has been demonstrated that transcript expression in blood cells recapitulates changes in gene expression seen in skeletal muscle [42]. A second area of investment is indicated for quantitative aspects of molecular techniques. Hence beyond the basic feasibility of measuring transcript and protein concentration in minute amounts of tissue, routine molecular tests have not established a generic standard or reference that would allow calculating effective concentrations. Another area of interest is the application of proton magnetic resonance spectroscopy of carnosine to draw conclusion on muscle composition [43], yet the robustness of conclusions from this determination needs to be validated.

In consequence minimally invasive means relying on biopsy sampling may be most promising in a setting that aims to develop, or refine, training protocols. We established with our studies that molecular parameters exist which reflect muscle performance and which can foretell the predisposition of exercised skeletal muscle to adapt to endurance training. All of these relate to metabolic fitness. This is shown for genotyping of the ACE I/D polymorphism based on mucosal samples, as well as for the quantification of changes in angiotensin 2, arterial oxygen saturation, serum lactate and glucose in blood serum after a maximal aerobic workout [28, 38, 41]. While the prospective power and robustness of these molecular candidates as biomarkers for muscle performance and its malleability remains to be evaluated; the current data indicate that the step from diagnosis to prognosis may be in close reach.

*Ethical considerations of muscle diagnostics*— It is now amply appreciated that physical performance is shaped by heritable (i.e. genetic) and environmental (i.e. training, nutrition) factors. Muscle malleability plays an important part in shaping this quality through its influence on motor function. Thereby the expression of a muscle phenotype and its reprogramming by training is reflected at the level of muscle gene expression [22]. These local adaptations can be readily assessed in samples of peripheral muscle tissue as collected with the percutaneous bioptic technique. Biopsy collection does not pose concern from the medical point of view when correctly performed [44] and is in routine use for diagnostic purposes of qualifying pathological samples [45]. From this standpoint, the diagnostic exploitation of bioptic material towards the tailoring of Sport performance is well supported.

1 As a matter of fact biopsies are now considered by prominent Sports institutions to  
2 complement classical physiological measures [46]. However, this benefit has – alike the one  
3 of genetic testing - to be weighed against possible negative effects. For instance, biopsies  
4 cause a moderate level pain due to muscle soreness which is not always compatible with more  
5 than recreational sports for a few days. As well (negative) discriminatory issues may be  
6 considered along with the fact that talent selection based on a default biological profile  
7 interferes with the concept of fairness which still is idealized in Sports. In this regard, an  
8 interesting lesson can be learned from the case of a former Olympic medalist in shot putter.  
9 This athlete, who was not affiliated with Germany, demonstrated an unusually high  
10 proportion of slow type fibers [47]. Therefore he likely would not have qualified for a talent  
11 program for strength type athletes as the default selection strategy for these types of exercise  
12 would have been based on a high content of fast fiber types. As shown from the unusual  
13 hypertrophy of the fast type fiber population in comparison to fellow competitors, his talent  
14 for explosive Sports was, however readily becoming apparent from the assessment of muscle  
15 plasticity to resistance type training. Thus a bioptic approach being targeted at the dynamic  
16 response to training, but not baseline measures from passive muscle and genetic  
17 predisposition alone, appears to offer the resolution to identify the skill for a high level  
18 athlete.  
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34 *Future challenges* –Given the spurt of interest in the discovery of biomarkers for  
35 successful endurance training it is likely that the modern Sports and Rehabilitation Clinics  
36 will see the entry of molecular tests before long [48-50]. These will allow screening with  
37 relative ease for constitutional factors affecting the activation of major pathways of  
38 adaptation. Yet these methods are not stand-alone and should be accompanied by relevant  
39 measures of functional variables to avoid false positive conclusions. This will be particularly  
40 attractive when tailoring training interventions and monitoring subject performance through  
41 the years.  
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53 *Conclusion* - Highly resolving molecular-biological tests targeted at the mechanism of  
54 phenotypic muscle plasticity appear on the horizon to quantify athletic potential and foretell  
55 its conditioning by training. A main topic in this regard is the quest to develop personalized  
56 protocols, which take different responsiveness of subjects into account. This may be achieved  
57 by quantifying malleability of skeletal muscle in a functional, whole body context, with a  
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combination of molecular tools and classical performance tests. In this regard a complementary approach monitoring the expression response in sampled muscle material in relation to major gene variants, which enhance the effect size of training, is tentatively identified to offer the power to predict the individual trainability of endurance performance. This stands and falls with the technical refinement of available methodologies and applied studies with trained populations to consolidate our knowledge on the underpinning adaptability of muscle's work capacity.

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Legends:

*Figure 1: Drawing illustrating the interrelationship between adaptations in organs that set endurance performance.*

The typical range of improvement for selected parameters related to endurance performance in untrained subjects after 6-10 weeks of endurance training is indicated.

*Figure 2: Adjustments of metabolic proteins after endurance training.*

A) Sketch of main metabolic pathways fueling ATP production during muscle work. Arrows indicate the flow of metabolic processes. Protein species being assessed are printed underlined in italics. B) Mean and standard error of fold adjustments in content of selected proteins after 30 endurance exercise sessions on a stationary bicycle. \* and + denote  $p < 0.05$  and  $0.05 \leq p < 0.10$  vs. values prior to training (paired T-test,  $n=8$ ). Abbreviations: AcCoA, Acetyl coenzyme A; ATP5A1, mitochondrial ATP synthase subunit alpha; B-OX, beta oxidation; CO I – CO V, complex I to complex V of the mitochondrial respiration chain; COX4; cytochrome c oxidase subunit 4; CS, citrate synthase; GLUT4, facilitative glucose transporter 4; HADH, 3-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase; NDUFA9, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9; SDH, succinate dehydrogenase; UQCRC1, subunit 1 of cytochrome b-c1 complex.

*Figure 3: The molecular response to endurance exercise highlights the responders to a given training scheme.*

A) Drawing illustrating the paradigm of gene expression. B) Illustration summarizing the protocol to quantify transcript expression after and endurance exercise test, and an example of possible results. Subjects completed a 0.5-hour bout of bicycle type exercise at 65% of peak aerobic power output. Fine needle biopsies were collected in alternate fashion from different locations of *vastus lateralis* muscle and subjected to the quantification of expression levels of 231 gene transcripts using a custom microarray. For details see Schmutz et al. (2006). Expression data were normalized and centered to the mean transcript signals of pre-exercise measures and subjected to cluster analysis to visualize expression profiles and map groups of transcripts that demonstrate co-regulation post exercise. This analysis illustrates that expression profiles 8-24 hours post exercise differ to the profiles seen prior and 1-hour post exercise. Expression signals are displayed in color-coding in a dendrogram. Examples of the mean values of change of up-regulated (red) and down-regulated (blue) transcripts of main gene ontologies related to aerobic myogenesis are shown to the right. Red and blue color reflects increased and reduced, respectively, transcript content relative to pre exercise levels. Arrowheads point to profiles visualizing an increased (red) and reduced (blue) molecular responsiveness between subjects. Abbreviations: COX4, cytochrome c oxidase subunit 4; ECHS1, Enoyl-CoA hydratase; HSL, hormone sensitive lipase; LPL, lipoprotein lipase; MEF2A, MEF2B, MEF2C; myocyte enhancer factor 2A, 2B and 2C, respectively; VLDLR, Very-low density lipoprotein receptor.

*Figure 4: Sketch illustrating a protocol to refine current tests of endurance performance with the inclusion of molecular tests.*

**Figure 1**

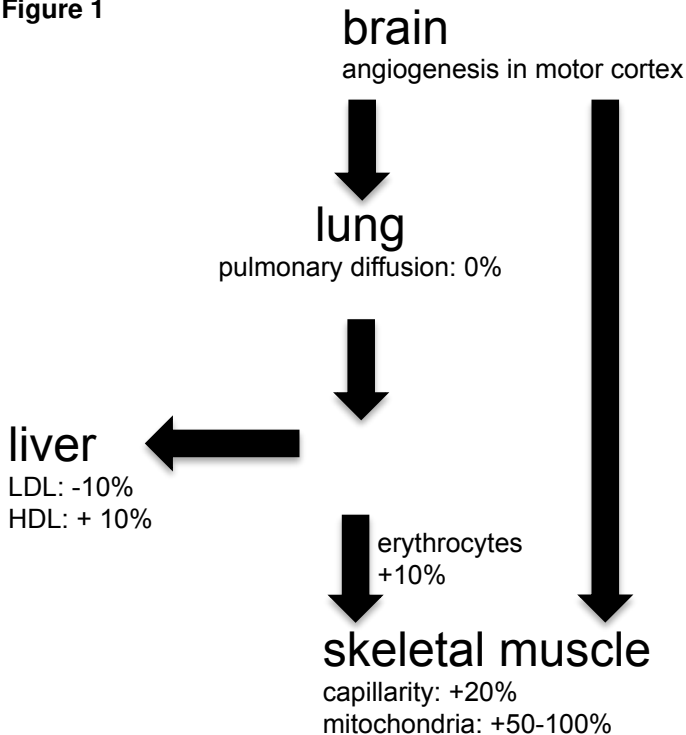
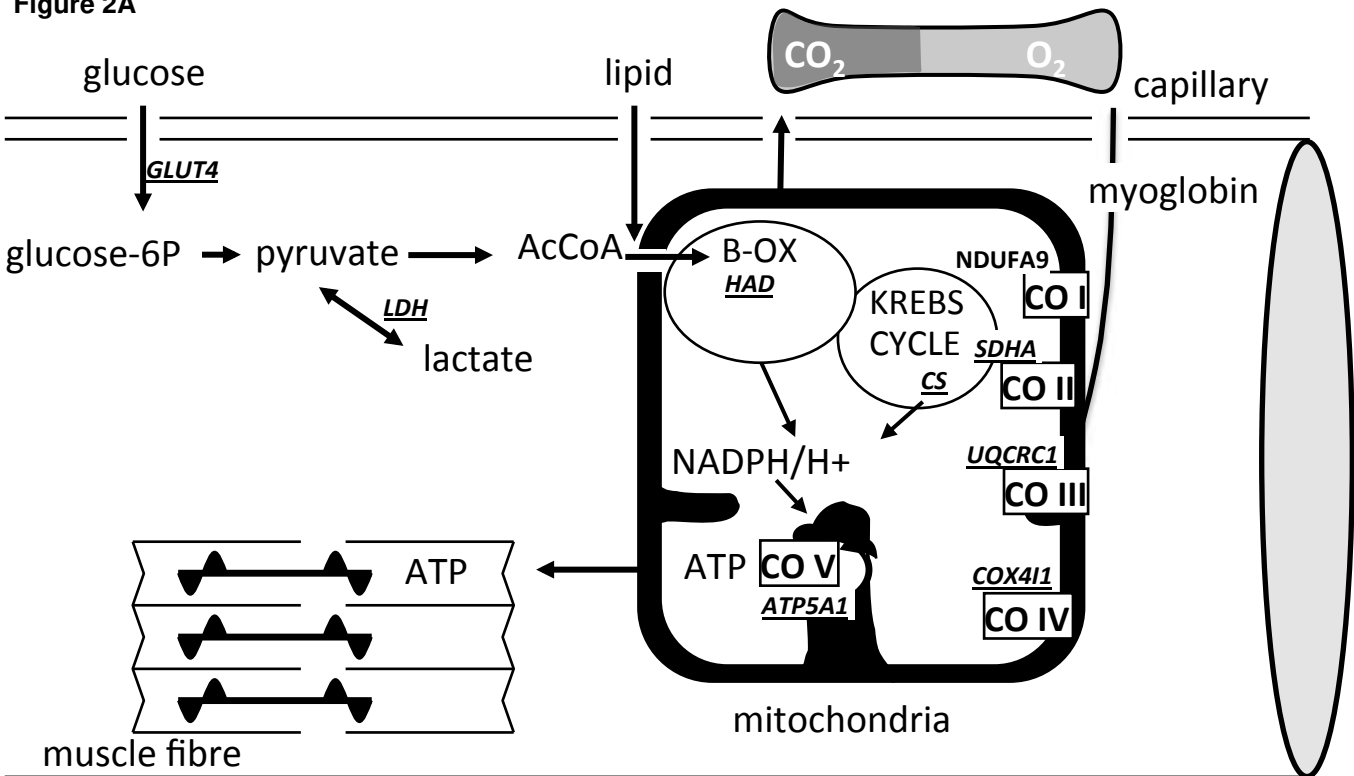
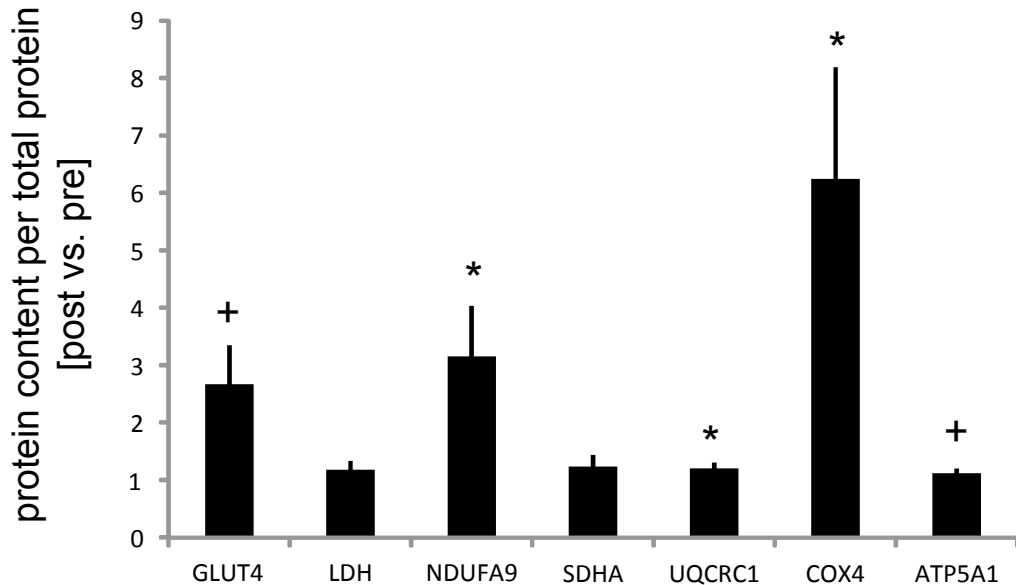


Figure 2A



**Figure 2B**



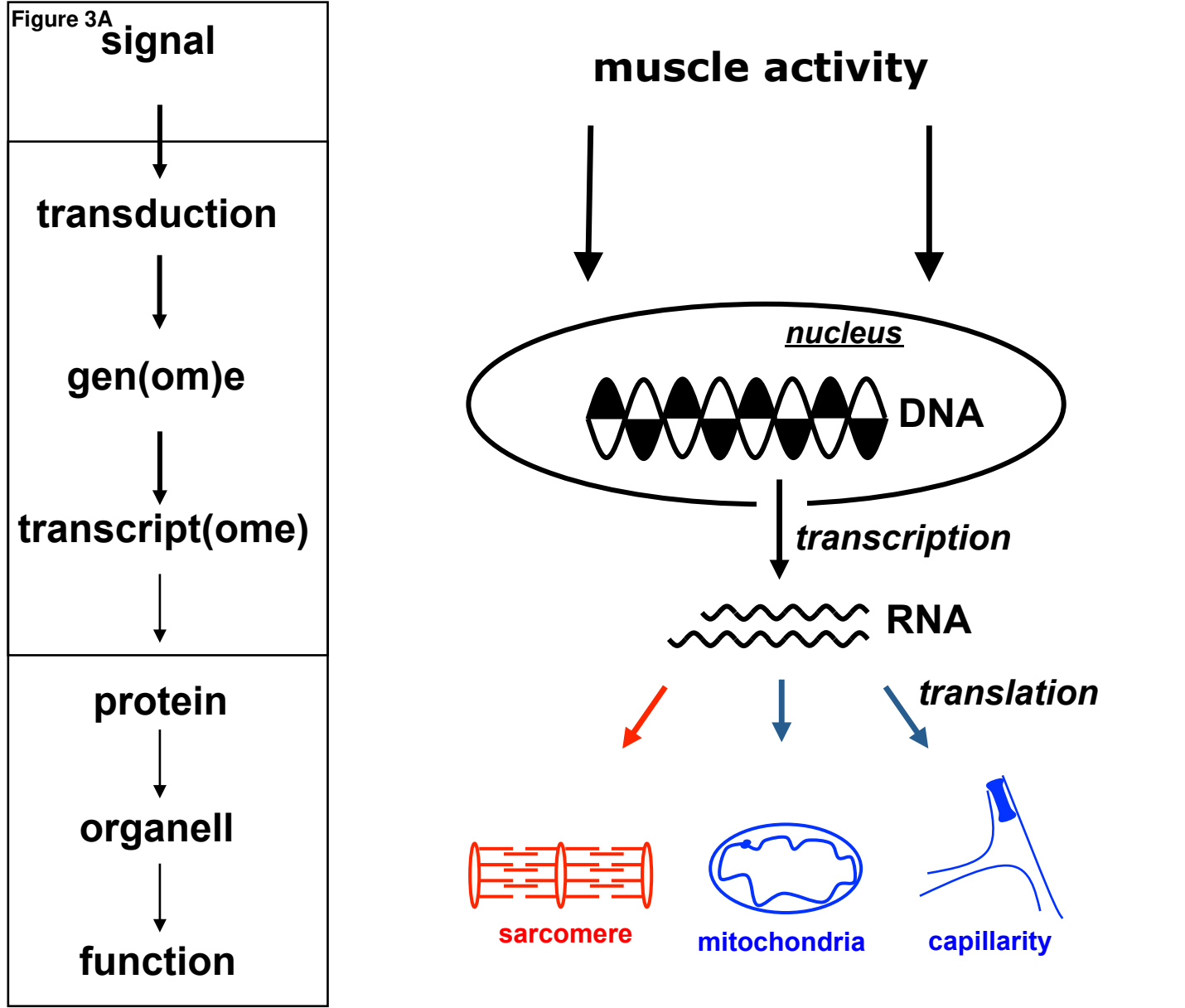


Figure 3B

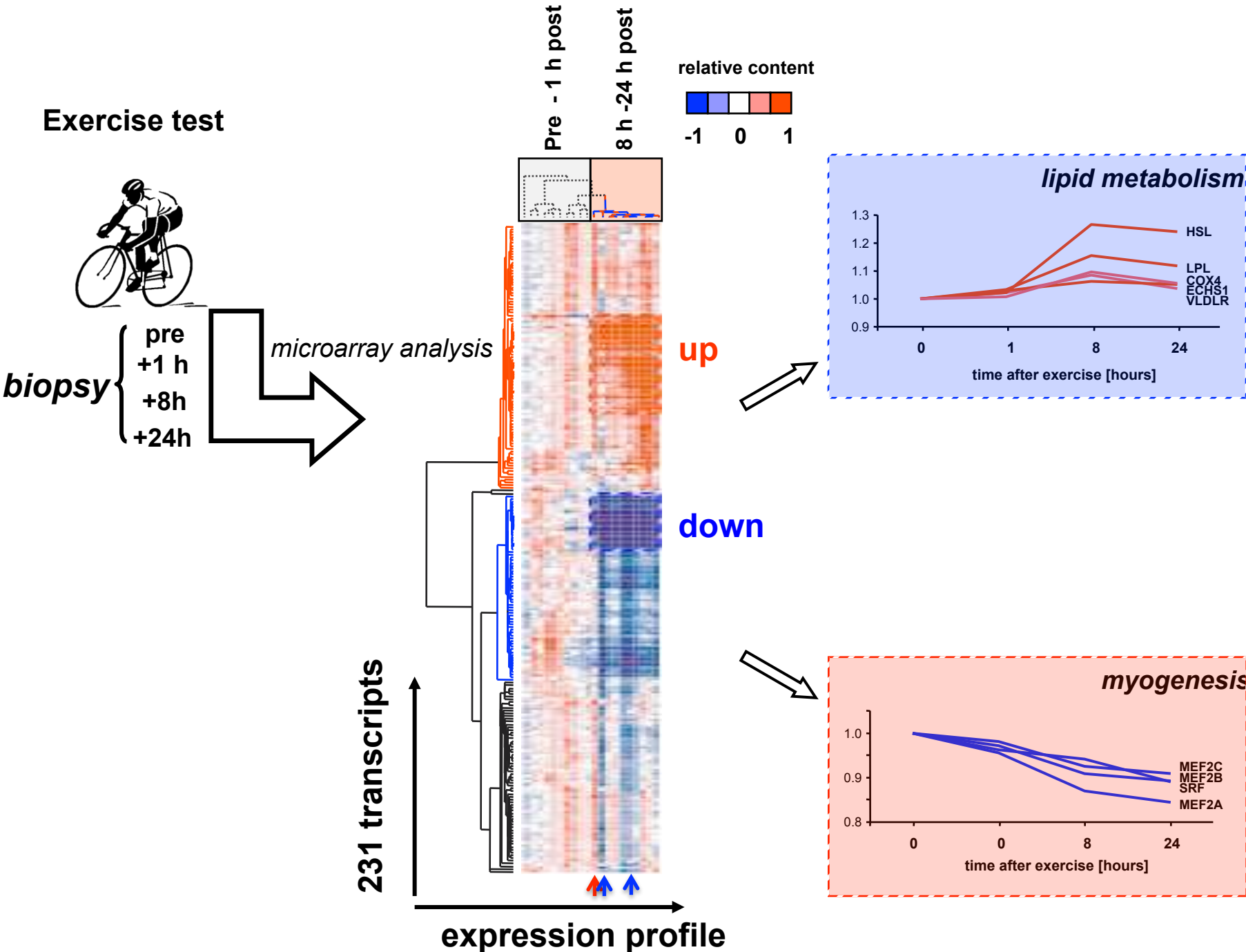
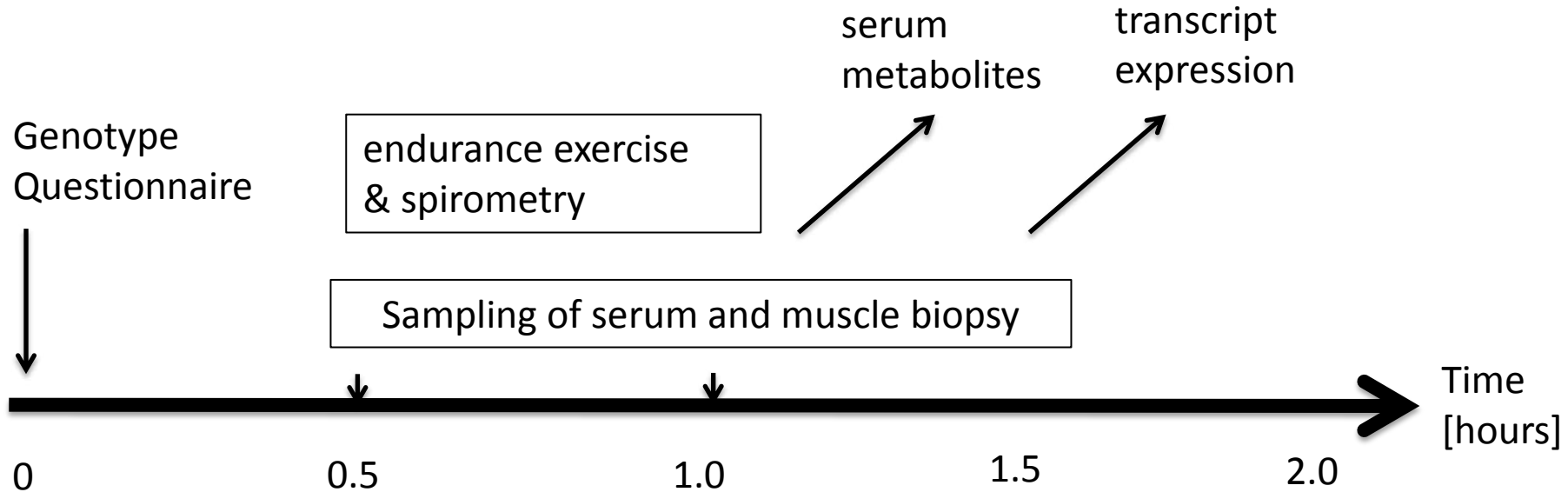


Figure 4





**Table 1: Features and drawbacks of standardized tests to assess parameters that affect endurance performance**

<i>Name</i>	<i>parameter being assessed</i>	<i>investment</i>	<i>advantage</i>	<i>limitation</i>
<i>generic tests:</i>				
anthropometric assessment	biometric data	30-minutes	simple measures	confounded by changes in economy
spiroergometry	VO2max test, respiration exchange ratio ventilation	1-hour	indications on aerobic substrate metabolism	
time-to-exhaustion	fatigue resistance	1-hour	specifically trained	variability
time trial	sustainable maximal velocity	1-hour	simulates competition reduced variability	subject motivation
<i>apprehended tests:</i>				
serum metabolites	concentration of lactate, pyruvate, acetoacetate, 3-hydroxybutyrate	15-minutes	minimally invasive	not always repeated post exercise
heart frequency	maximal heart rate during exercise	30-minutes	precise training indications location independent	objectivity, perturbation through environmental variables
strength and power test	force, power, torque	15-minutess	assessment of functional parameters	standardization of measurement position
<i>Further characterization:</i>				
health screening	blood pressure, nutrition	30-minutes	assessment of body homeostasis	representativeness often not verified
questionnaire (SF-36 or other)	well-being and lifestyle	15-minutes	sampling of environmental and psychological variables	parameters often not numerical